FUNGAL DIVERSITY IN AND AROUND TERMITE MOUND, MOUND MATERIAL ANALYSIS, AND RESPONSE OF SUBTERRANEAN TERMITE, *Globitermes sulphureus* (Haviland) (BLATTODEA: TERMITIDAE) TO FUNGI

By

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KEPELBAGAIAN FUNGI DI DALAM DAN SEKELILING BUSUT ANAI-ANAI, ANALISIS BAHAN BUSUT, DAN RESPON ANAI-ANAI TANAH, *Globitermes sulphureus* (Haviland) (BLATTODEA: TERMITIDAE) KEPADA FUNGI

ABSTRAK

Kini, ramai saintis cuba mengkaji kaedah kawalan biologi dengan menggunakan kulat sebagai agen untuk mengawal serangga perosak termasuk anai-anai tanah. Kajian ini bertujuan mendapatkan kefahaman yang mendalam terhadap interaksi anai-anai dan kulat berdasarkan kondisi seperti yang tertera di atas. Pengasingan, penulenan, dan identifikasi kepelbagian kulat di dalam busut anai-anai *Globitermes sulphureus* telah dijalankan. Berikutan itu, kulat tersebut diperkenalkan kepada anai-anai dan gerak balas anai-anai terhadap kulat dikaji. Analisis lanjutan menggunakan bahan busut menunjukan kepelbagaian kulat di dalam busut anai-anai *G. sulphureus*. Kajian awal menunjukkan kehadiran 24 spesis kulat yang diisolasikan daripada sepuluh lokasi daripada busut *G. sulphureus*. *Trichoderma sp.*, *Aspergilus sp.*, dan *Penicilium sp.* merupakan antara kulat yang kerap ditemui di dalam busut anai-anai. Ini menunjukkan bahawa anai-anai tanah mengamalkan hubungan simbiosis dengan kelima-lima spesis kulat tanah yang ditemui wujud seiringan dengan anai-anai tersebut. Akhir sekali, kajian anti-kulat menggunakan kandungan usus *G. sulphureus* menunjukkan kehadiran bahan anti-kulat berikutan keupayaanya untuk membantut pertumbuhan kulat, terbukti melalui pembentukan suatu zon jernih di sekitar kawasan aplikasi kandungan usus tersebut.

Kekunci: kulat tanah, *Globitermes sulphureus*, anti-kulat

FUNGAL DIVERSITY IN AND AROUND TERMITE MOUND, MOUND MATERIAL ANALYSIS, AND RESPONSE OF SUBTERRANEAN TERMITE, *Globitermes sulphureus* (Haviland) (BLATTODEA: TERMITIDAE) TO FUNGI

ABSTRACT

Nowadays, many researchers are trying to develop biological control methods using fungi to control insect pests including termites. This study explored the termite-fungus relationship using *Globitermes sulphureus* (Haviland) as the model species. Isolation, purification and identification of fungi diversity in termite mounds of *G. sulphureus* werw carried out. These followed by introducing the fungus to termite and identified the termite interaction with the fungus. Further analysis of termite nest carton showed fungi diversity of *G. sulphureus'* mound. Preliminary study found 24 species of fungus that were isolated and purified from ten different locations of *G. sulphureus'* mound. *Trichoderma* sp., *Aspergilus* sp., and *Penicillium* sp. are the fungal that are commonly encountered in termite mounds. It is revealed that termite practice symbiont relationship with the five species of soil fungi that has been found with them. Lastly, the anti fungal test from *G. sulphureus'* gut showed that they have anti fungal compound since it has the ability to inhibit the growth of fungus, shown by the formation of a clear zone surrounding the termite's gut.

Keywords: soil fungus, *Globitermes sulphureus*, anti fungal

CHAPTER ONE

INTRODUCTION

Termites are regarded as pests related to cockroaches'. Since termite food is mainly wood and woody tissue, this insect causes many problems to the wood users. Most species of termites feed on dead-plant materials above, at, or under soil surface to perform a vital ecological role in litter breakdown and recycling of the mineral elements (Lavele and Spain, 2001), including dead foliage of grasses and other types of vegetation, woody materials such as roots and seeds, faeces of higher animals and other materials. As a social insect, termites build nest to protect their entire colony from extreme microclimate and to defend against predators. In some species of termite, nest is functions as food storage (Noirot, 1959 and Thorne et al., 1996). Noirot and Johana (2000) grouped the termite nest into three categories, i. e., subterranean nest (below ground level), epigeal nest (above the soil) and arboreal nest (on trunk or branch tree and connected to the soil by crating mud tubes).

Globitermes sulphureus (Haviland) is commonly found in Malaysia, Singapore and Vietnam. It has been classified under family Termitidae. This species is easily identified based on the bright yellow colored abdomens of the soldiers (Lee *at al.*, 2003). *Globitermes sulphureusi*, highly prevalent in Malaysia, is a mound building termite, which builds the epigeal nest. Noirot (1959) has categorized the mound of *G. sulphureus* into five layers, i.e., royal chamber, pressed wood areas, internal zone of the wall, external layer of the wall and outer portion (bark). They forage in the sand, feed on dead wood, sometimes they are found attacking living trees and buildings (Roonwal, 1970).

However, *G. sulphureus* is also beneficial to the environment as they break down the wood debris and return them to the soil. Besides termites, other microorganisms such as fungi also play an important role in decomposing dead wood and grasses. There are three functional groups of fungi: decomposer, mutualists and pathogen. Around 100,000 out of 1.5 million estimated fungal species have been described by taxonomist (Hawksworth, 1995). The microbial populations in the soil are very diverse. Torsvik *et al.* (1996) calculated the presence of approximately 6,000 different bacterial genomes per gram of soil by taking the genome size of *Escherichia coli* (Migula) Castellani and Chalmers as a unit.

Being subterranean, *G. sulphureus* are exposed to various fungi and other pathogens that are abundant in the soil. Many studies regarding the interactions between termite and soil fungi have been conducted (Sand, W.A., 1970; Wong and Cheok, 2001; Rosengaus *et al*., 2003). Wong and Cheok (2001) reported that relationship between termite and fungi could be either symbiotic, attractant, antagonistic, pathogenic or parasitic. Amburgey (1970) found more than 50 species of brown-rot fungi interacted with subterranean termites. According to Hendee (1953), fungi are able to modify the nutrition, nest construction, survival and caste development of the termites.

Most literatures discuss about the relationship between termite and fungus growing termite (*Termitomyces*). Fungal epizootics to termite populations are not well defined yet. This study was initiated to explore the interactions between *G. sulphureus* and fungi. The objectives of this study are:

a. To determine the diversity of fungi in the termite's nest/mound (inside, outside and nearest/adjacent soil).

b. To observe the termite behavior when coming into contact with soil fungi.

c. To determine whether termites produce secretion with anti-fungal activity.

CHAPTER TWO

LITERATURE REVIEW

2.1. Biology of *Globitermes sulphureus* Haviland

Termites are eusocial insects, classified under family Blattodea under epifamily Termitidae (Inward *et al.*, 2007). There are now 4,000 species known, 2,600 species of which were taxonomically described belonging to 281 genera and only 10% of them have been reported as insect pests. Termites are classified into a few groups based on their habitat, life types and feeding types. There are three types of termites based on the habitat, i.e. drywood termites (e. g. family Kalotermitidae), dampwood termite (e. g. family Termopsidae) and subterranean termites (e. g. Termitidae). One-piece nesting such as family Termopsidae and multiple-piece nesting termite such as Mastotermitidae and Termitidae are grouped according to the life types. Based on feeding types (mandibles morphology and gut content), termites can be categorized into soil-feeders, soil/wood interface-feeders, wood feeders, litter-foragers and grass feeders.

Termites undergo incomplete metamorphosis (hemimetabolous) that is different from other social Hymenoptera (Krishna, 1969). These societies contain males and females although males do not play any role in the colony (Krishna, 1969). Termite societies are diploid, while Hymenoptera are haplodiploid (Korb, 2007). A termite colony basically comprises of reproductives, alates, workers, soldiers and immature individuals. Workers are the largest number in the colony and they are wingless, blind and sterile/not sexually mature. Generally, workers are assigned all kind of works e.g. feeding other castes, grooming the queen, making tunnels, and excavating the nest (Weesner, 1969).

Soldiers are the second largest number in the colony comprising 5-10% of the entire active colony. This caste has large jaws, big head and they usually gather at the front to protect the colony from invader, predator or other insects such as ants and other termites species (Weesner, 1969). Some species of termites have two sizes of soldiers; major and minor soldiers. Immature individuals are recognized as nymphs which posses a pair of wing buds which potentially develop into winged alates; while larvae lack wing buds and display several moulting with little morphological changes to either workers or soldiers (Pearce, 1997). Nymphs moult into sexually mature, high pigmented and winged adult males and females (alates). Alates are produced on a seasonal basis, swarm under suitable temperature, humidity, light condition and are potential founders of new colonies. After a short flight, males and females will get paired. Each pair will burrow in the soil or wood (Kalotermitidae) and makes a small nest in which they mate and lay eggs.

Secondary reproductives in termite colonies are referred to an any reproductive individual that is not derived from primary pair(s) (Myles, 1999). Secondary reproductives can develop from immature apterous and brachypterous castes of termites through one of the various potential developmental pathway. There castes will replace main reproductives when the pairs become moribund, die or have low egg production, to ensure the colony's lifespan continues, while supplementary reproductives coexist with the primary reproductives in satellite colony during budding process.

Genus *Globitermes* consists of four species and subspecies: namely; *G. globosus* Haviland from Borneo, *G. depilis* Holmgren from Sarawak in Borneo, G. *vadaensis* Kemner from East Java and *G. sulphureus* Haviland from the Indo-Malayan region (Roonwal, 1959). *Globitermes sulphureus* is widely distributed in Burma, Vietnam, Thailand, Cambodia and Peninsular Malaysia. The colony of *G. sulphureus* contains tens of thousands of individuals where 5 to 10% of the active individuals represent the soldier (Bordereau at al., 1997).

Globitermes sulphureus can be identified based on the bright yellow coloured abdomens of the soldier (Plate 2.1) (Bordereau *et al*., 1997; Lee *et al*., 2003). The yellow colour liquid in their abdomen is a defensive secretion, which occupies a large part of the abdominal cavity and in a gland of the thorax (Bordereau *et al*., 1997). The soldiers secrete the yellow liquid through their mouth when they fight with the enemies to defend the colonies. The yellow liquid dries rather quickly when it comes into contact with air and produces a sticky substance that binds to the enemies such as ants and other termite species (Bodereau et al., 1997). The soldiers of *G. sulphureus* are equipped in the two large curve mandibles in the form of a lashing-piercing type. Workers of *G. sulphureus* also actively attacked the enemies (John, 1925; Bathellier, 1927).

Plate 2.1. Soldier of *G. sulphureus* (Haviland)

Globitermes sulphureus builds a dome-shape mound that may reach 100 cm in height and 65 cm in diameter. The characteristics of *G. sulphureus* mound is different from other Amitermitinae mound. The mound constructed by this species is unique and complex. It can be divided into 5 layers. The first layer is a central cavity of the mound that fills up with balls or pressed. This area is surrounded by laminated contraction (second layer). The royal chamber is located at the centre and almost at the base of the mound (third layer). *Globitermes sulphureus'* mound is covered with two layers of wall, i.e., internal (fourth layer) and external wall (fifth layer). There is a space that separates the wall from the bark. The central part of the mound contains termite eggs, young larvae and workers. Based on the chemical analysis of *G. sulphureus'* nest carton, termite utilizes some of the undigested food from the internal regions of the nest while the proportion of soil material and stercoral elements vary almost continuously from the exterior to the interior of the nest (Noirot, 1959). The development of caste takes places as in *Macrotermes gilvus* Hagen and its swarm occurs in January and May (Roonwal, 1959).

Globitermes sulphureus is highly prevalent in Malaysia and forages on dead wood, and sometimes also attacks living trees and buildings (Krishna and Weesner, 1970). Harris (1971) reported that *G. sulphureus* is one of the important termite pests in agriculture plantation such as coconut and oil palm plantations.

2.2. Soil Fungi

Soil consists of layers of mineral with different thickness, water, gases and organic substances in the solid, gaseous and aqueous states (Birkeland, 1999; Voroney, 2006; Brady and Well, 2007). Soil plays important roles in the ecosystem such as medium for plant growth, systems for water supply, recycling system for nutrients and organic wastes, engineering medium for soil organisms, physical and cultural heritage and platforms for human structures (Blum and Eijaskers, 1993; Brady and Well, 2007). Presence of soil organisms is important in maintaining the fertility, structure, drainage, and aeration of soil. Soil organisms are referring to the creatures that live in the soil.

Brady and Well (2007) classified soil organism based on the organism size, i.e., (1) macrofauna (>2mm); all the heterotrophs, largely herbivores and detritivores, (2) macroflora; largely autotrophs, (3) mesofauna (0.1 – 2 mm); all the heterotrophs, largely detritivores and predators, (4) microfauna (<0.1 mm); detrivores, predators, fungivores, bacterivores, (5) microflora (<0.1 mm); largely autotrophs, heterotrophs, heterotrophs and autotrophs. One of the most important roles of soil organisms is to break up the complex substances in decaying plants and animals in order to be used again by living plants. This involves soil organisms as catalysts in a number of natural cycles, among the most prominent being the carbon, nitrogen, and sulphur cycles.

There are about 1.5 million species of fungi found in the soil. However, only 5-10% of them are taxonomically identified (Hawksworth, 1991; Roane *et al*., 2009). Fungi are microscopic cells which are part of eukaryotic organisms that is classified in one kingdom called fungi, separated from bacteria, protozoa, chromista, plantae and animalia (Caraller and Smith, 1998). Being abundant throughout the world, most fungi are not striking because of the small size of their structures, and their cryptic lifestyle in the land, on in-animate objects, and as symbiont of plants, animals or other fungi. They become noticeable during fruiting, either as mushrooms or molds.

Fungi perform important roles in the organic material decomposition and in nutrient cycling and exchange (Warcup, 1951; Wainwright, 1988; Haawksworth *et al*., 1995).

Based on the taxonomic groups the major phyla (division) of fungi have been classified mainly on the basic of characteristic of their sexsual reproductives structures. Curently seven phyla are proposed: (1) Microsporidia, (2) Chytridiomycota, (3) Blastocladiomycota, (4) Neocallimastigomycota, (5) Glumeromycota, (6) Ascomycota and (7) Basidiomycota (Hibbert *et al*. 2007). Based on moacroscopic descriptions, fungi can be divided into three groups; (1) yeast, (2) mold and (3) mushroom (Brady and Well, 2007; Roane *et al*., 2009). Yeasts are unicellular fungi, which are able to ferment and can cause candidiasis of the skin or mucous membranes. They can be isolated from fruit, soil, aquatic environments and normal microbiota of animals. Molds are filamentous fungi that are common in spoiled food, soil and crops that grow in masses to form hypae and mycelia. Mushrooms are part of Basidiomycota and Agaricomycetes, which are also known as fleshy and spore-bearing fruiting body of fungus. They are commonly found above ground such as dead trees, plat materials and soil (Brady and Well, 2007; Roane *et al*., 2009).

Soil fungi haves several functions such as biotransformation, nutrient cycling, disease suppression and water dynamics. They are also useful as metabolites for plant growth, enzymes activity, biological control for insects and nematodes, and associations with mycorrhizae in forestry (Alexopoulos *et al*., 1996; Jenkins, 2005; Murphy *et al*., 2007). Jenkins (2005) and Murphy *et al*. (2007) divided fungi into three groups based on their functions: (1) decomposers due to their ability to degrade organic matter into organic acids, carbon dioxide and fungal biomass and also to break down the highly resistant components e.g. cellulose, protein and lignin; (2) mutualists as they evolve profitable relationship with plants since they help the plants to obtain nutrients from soil and also protect the plant roots from pests and pathogens and the common group of these is mycorrhiza (arbuscular, ectomycorrhizal, ericoid and orchid); (3) pathogens, the most dominant group in soil, cause nutrient deficiency in plant and death as they penetrate plant body, decompose living tissue and weaken the plants and examples of these fungi are *Verticillium*, *Phytophthora*, *Rhizoctonia* and *Pythium*.

2.3. Termite – Fungus Relationship

The most interesting of the fungus-insect symbiotic relationships are those involving colonial insects. One of the most important driving forces that result in symbiotic relationships between microorganisms is the inability of animals to digest cellulose. Herbivores, such as horses, sheep, cows, goats, etc., do not actually have the ability to digest the cellulose from the plant material that they consume. Instead, they have symbiotic bacteria, in their stomach, that have the cellulolytic enzymes that digest the plant material for them. Other animals, such as detritivores, do not carry microorganisms in their gut, but rather consume mycelium in well-decomposed plant material as their food source. Thus, symbiotic relationships between animals and various microorganisms are common.

The other side of symbiosis is happening between insect and fungi. Interaction between insect and fungi has been widely described. From million of fungal species that has been discovered, 500,000 species are reported to have association with insects (Driver and Milner, 1998). One of them is the interaction between fungi and termites. Fungi was the first organism to conquer wood when it is exposed to the soil (Butcher, 1968) and termite will come after that and compete with fungi for available nutrients and moisture (Sands, 1969), and competition for resources between termites and fungi may lead to habitat partitioning, symbiosis, or pathogenic relationship.

2.3.1 Termite-fungal symbiotic relationship

Colonies of fungus-growing macrotermitine termites and attine ants are among the most impressive animal phenomena in the world. They can have nest volumes of thousands of liters, may persist for decades, and contain millions of sterile helper individuals, which are normally the offspring of a single queen (Hőlldobler, B. & Wilson, E. O.,1990; Shellman-Reeve, J. S., 1997). The agricultural symbiosis with fungi has allowed both the ants and the termites to occupy previously inaccessible niches that have abundant resources (Waller, D. A., 1988). The phylogenetically most derived genera of the attine ants have become dominant herbivores of the New World tropics (Waller, D. A., 1988). Analogously, the fungus-growing termites have become major decomposers of the Old World tropics (Bignell and Eggleton, 2000) and form perhaps the most complex colony and mound structures of any invertebrate group. The two main symbioses of social insects with fungi are similar in many respects, but they differ in others. The fungal symbionts of the attine ants rarely fruit and are normally propagated clonally and vertically by dispersing queens (Mueller *et al*., 2001 and Green *et al*., 2002). In contrast, the symbionts of the Macrotermitinae often produce sexual fruiting bodies such that horizontal acquisition of symbionts has been inferred to be the rule, although exceptions do occur (Johnson *et al*., 1981; Darlington, 1994, and Katoh *et al*. 2001). Recent studies have broadened our understanding of the evolution of the symbiosis between the attine ants and their fungi considerably (Mueller *et al*., 2001; Green *et* *al*., 2002; Chapela *et al.*, 1994; Mueller *et al.* 1998; Currie *et al*. 1999; and Mueller, 2002), but similar large-scale studies of the Macrotermitinae and their *Termitomyces* symbionts have been lacking.

Symbiotic relationships have had an essential role in termite evolution and involve a range of intestinal microorganisms including protists, methanogenic Archaea, and bacteria (Bignell, 2000). However, only a single Termitidae subfamily, the Macrotermitinae, has evolved a mutualistic ectosymbiosis with fungi of the genus *Termitomyces* tri e Termitomyceteae (u lich Singer, family Tricholomataceae Roze, Basidiomycotina]. The fungus helps the termites to degrade the plant-derived material (e.g., wood, dry grass, and leaf litter) on which they live (Johnson *et al*., 1981). It grows on a special structure in the nest, the fungus comb, maintained by the termites through continuous addition of predigested plant substrate while the older comb material is consumed (Rouland, 2000).

Macrotermes colonies host a remarkable symbiotic relationship with a basidiomycete fungus, *Termitomyces.* The termites cultivate the fungi in a fungus garden, comprising a few hundred fungus combs, structures built from chewed up grass and wood, and inoculated with fungal spores. Each year, these fungi produce a crop of large mushrooms, known locally as *omajowa*, which are highly prized as a delicacy. Unlike the fungi cultivated by leaf-cutter ants, which the ant colony uses as food, the *Termitomyces* culture in a *Macrotermes* nest aids in the breakdown of cellulose and lignin into a more nutritious compost which serves as the termites actual food. The fungus garden is, therefore, a kind of extracorporeal digestive system, to which termites have 'outsourced' cellulose digestion.

The fungi also play a significant role in the social homeostasis in

Macrotermes colonies, in particular aiding in water balance for the colony. This has made *Macrotermes* colonies much more tolerant of dry conditions than other termites, which enables them to exist in dryer environments than termites are commonly found. The fungi are part of an extracorporeal digestive system that converts undigested woody material in plants into higher quality oligosaccharides and more easily digestible complex sugars. There is some nitrogen fixation that also takes place. The fungi are grown in structures called fungus combs. Combs are made from macerated woody material, gathered by foraging workers, that is chewed up and swallowed. When the foragers return to the nest, they evacuate this material very quickly as pseudofeces, passing it on to nest workers, which take this material and mold it into the fungus comb.

Somewhere along the way, perhaps in the digestive tracts of foragers or nest workers, this woody slurry is inoculated with a variety of fungal spores. Once deposited in the comb, the Termitomyces spores germinate and begin spreading hyphae through the comb. As these grow, they delignifiy and digest cellulose, converting it to simpler sugars and nitrogen. The termites then consume this enriched fodder for food. The structure of the combs is dynamic. Fresh material is continually added to the top, and digested material is consumed from the bottom. Food "flows through" the comb, just as silage flows through a silo.

A colony amasses a large number of fungus combs, gathered into a series of galleries atop the nest called a fungus garden. The collection of fungus combs in the photograph to the right represents a small sample of a single colony's fungus garden. Each fungus comb is placed in a semi-enclosed space called a gallery. The total mass of fungus combs typically exceeds the colony's entire mass of termites by about eight fold - roughly 25-40 kg of fungus comb per colony.

2.3.2 Biological control of termite.

Termite is a social insect that is live in the soil with high population density under humid condition which is suitable for fungal to growth. Soils contain millions of fungi species and most of fungi are entomopathogen. The present of entomopathogenic fungi in the soil will give an effect into termite colony. Based on this fact, many reseach has been done to develop and utilize the use of entomopathogenic fungi as a biological control of termite.

Biological control constitutes a more environmentally acceptable alternative to traditional chemical control measures. When successfully implemented, it can yield permanent, cost-effective management of pest populations with minimal environmental disturbance (Culliney and Grace, 2000). Classical biological control programmes (i.e. the discovery, release, and colonization of natural enemies: arthropod predators and parasitoids, and pathogens) have proved successful against a variety of pests, and knowledge of the role natural enemies play in the ecology of these species has steadily accumulated (Huffaker & Messenger, 1976; DeBach & Rosen, 1991). However, data for termites are relatively scant. Although the influence of predation on termite distribution, abundance and population dynamics may be partially understood in a few cases (Wood & Johnson, 1986), the natural role of termite pathogens is much more obscure (Lee & Wood, 1971).

Much research interest, especially in recent years, has focused on the use of fungal agents for pest control (Ferron, 1978, Kartika *et al*. 2006, Guswenrivo *et al*.2007, Rahimzaleh *et al*., 2012 and Cheraghi *et al*., 2013). At least 22 species of fungi are obligate ectoparasites of termites (Blackwell & Rossi, 1986), although their biological control potential apparently has not been evaluated. It is the endoparasitic fungi, however, that have perhaps the greater potential for biological control of termites, and these have received more attention by researchers (Culliney and Grace, 2000). Only two fungal pathogens, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hyphomycetales), have been extensively evaluated for termite control (Culliney and Grace, 2000) and recently also *Paecilomyces fumosoroseus* (Wright *et al*. 2005)

Several laboratory studies have examined effects of *M. anisopliae* and *B. bassiana* on subterranean termite populations. Results have varied with the termite species and strain of fungus tested, and the concentration of inoculum and its mode of delivery to termite populations (Sajap & Kaur, 1990, Zoberi, 1995, Yanagawa *et al.* 2008 and Chouvenc and Su, 2012). Spread of the disease through colonies was complete, even though some of the fungus-killed termites were buried by nest mates, impairing growth of the fungus and preventing dispersal of conidia. Results suggested that epizootics within natural subterranean colonies might be initiated by a sufficient number of vector termites contaminated with large doses of inoculum introduced directly into the soil (Culliney and Grace, 2000). Other laboratory studies of baits have assessed the infectivity of sporulating strains of *B. bassiana* and *M. anisopliae* to *C. formosanus* and it came with suggested result that fungal bait stations might be useful in termite control by providing a continuous and non-repellent source of sporulating cultures for foraging termites to contact, although a self-perpetuating infection within the experimental colonies was not demonstrated (Grace, 1993).

Subsequent field experiment did not give a good result of controlling termites (Hänel & Watson, 1983). This was due to the complexity of the problems faced when these entomopathogenic fungi are applied in the field. But lately, Milner

(2003) reported that by using large quantity of pure dry conidia *M. anisopliae* and blown it directly into the mound of *Coptotermes* sp. and *Nasutitermes* sp., can be destroyed the termite colonies. It has shown too by Lenz (1996) and Lenz *et al.* (2000) at different environtment. It said that applications of *M. anisopliae* can be eliminated the colonies of *Neotermes* sp. easily.

In other hand, researches on termites biocontrol was developet. The obsevation of the changes of termites behavior, the activity of termite and the mortality of termites when it came to contact with entomopathogenic fungis were kept developt (Yanagawa *et al.* 2008, Chouvenc and Su, 2010, Yanagawa *et al.* 2011a and Yanagawa *et al*. 2011b). Overall, the fungi would seem to offer the most potential for at least some measure of termite control. However, the use of fungi in control programmes is compromised by inherent biological limitations and logistical problems. Fungi have a slow mode of action and require high levels of relative humidity; there is also the need for large quantities of infective conidia to contact the target pest population in order to yield an acceptable level of control (McCoy, 1990). It has also been suggested that, because so few of the many strains evaluated for termite control were effective, even under favourable conditions, termites may have evolved a degree of resistance to fungal pathogens (Burges, 1981). However, there is as yet no good evidence to support this speculation.

Perhaps because of the many difficulties involved with their use, there are, at present, few commercial fungal preparations available for pest control in the USA. Further development for biological pest control of *B. bassiana* and *M. anisopliae,* in particular, continues to be hampered by a lack of cost-effective methods for mass production (Federici, 1990). Fungi may yet find their most effective use in the form of baits, and research in this area is progressing (Delate *et al.,* 1995; Jones *et al.,* 1996; Staples & Milner, 1996). Effective baiting schemes for termite control require delivery of sufficient spore inoculum to active termites and subsequent transfer from them to nest mates without stimulating colony defensive behaviours. This will entail both improvement of field techniques for dispensing conidia and identification of new fungal species and strains capable of initiating and sustaining an epizootic once they are introduced into the nest (Culliney and Grace, 2000).

CHAPTER THREE

MARERIAL ANALYSIS AND THE SURROUNDING SOILS OF THE MOUND OF SUBTERRANEAN TERMITE, *Globitermes sulphureus* Haviland

3.1. Introduction

Termites are serious pest in urban, agricultural and forest environments in Malaysia (Lee *et al*., 1999). Lee *et al*. (2002) reported that termites contributed to approximately 50% of business turnover of the pest control industry in Malaysia. Many studies on termites have been conducted in Malaysia where it was pioneered by Haviland in year 1898. In 1913, Bugion created a list of Indo-Malayan termites, and in 1925, John worked on termites of Ceylon, Peninsular Malaysia, Sumatra and Java (Lee, 2002). A total of 175 species of termites were recorded in Malaysia and 5 species among them caused 90% damages in Malaysia (Tho, 1991; Kirton and Wong, 2001; Lee *et al*., 2004; Lee, 2002b; Lee *et al*., 2003).

Termite are social insects, living in colonies and making nests that are located in the ground (subterranean), above ground (mound building termite), in a tree, and inside wood. Termite nest is one of the most complex and amazing buildings made by animal. They are able to build a nest that attain sizes of two or four orders in magnitude than their size (Grassé, 1984). Beside that, the population of termites will also give an impact on the size of the nest. Lee (2012) proved that the size of *Macrotermes gilvus* mound correlated with the population of the termite colony. It has also been proved by Darlington and Dransfield (1987), Lepage and Darlington (2000) with the termites *M. michaelseni*. Generally, termite nests have a unique structure which contributes in mantaining homeostatic balance of the environment, protection of the colony from predators, source of all the colony activity and colony's defence against disease (Lüscher, 1955; Kor and Linsenmair, 1999, 2000; Korb, 2003; Dejean and Ruelle, 1995; Dejean *et al* 1996, 1997; Fank and Tofts 1994, Traniello and Rosengaus, 1997, Pie *et al.* 2004).

The characteristic of termite nest has been investigated by Perna *et al.* (2007) in the genus *Cubitermes*. Perna et al. (2007) reported that *Cubitermes* builds a very well interconnected network inside the nest and it enhances defense against predators. Besides that, many studies on lower termite conducted by many researchers that analysed nutrients content in termite mounds and soil from around the mounds (Lee and Wood 1971; Abbadie and Lepage, 1989; Lavelle *et. al.*, 1994). In addition, Bezerra-Gusmão (2011) reported that the proportion of carbon inside *Constrictotermes cyphergaster* nest formation contributes to the carbon cycling in dry semiarid area.

This study was conducted to measure the nutrient and organic matter of the nesting material of *G. sulphureus*, which belong to the family Termitidae and subfamily Amitermitinae. They are highly destructive to dead wood and sometimes to living trees (Krishna and Weesner, 1970). *Globitermes sulphureus* is one of the destructive termite pests in Malaysia and cause damages to buildings and trees. It can be found at Indo-Malaya region. It can be identified based on the bright yellow colored abdomen of the soldiers (Lee *et al*., 2003). Harris (1971) reported that *G. sulphureus* is one of the important termite pests in agricultural plantations such as coconut and oil palm. Limited information about the ecology of *G. sulphureus* causes intricacy to pest control operators in handling and controlling the termite.

In addition, *G. sulphureus* co-exist and infests buildings and structures. Lee (2007) has stated that paper-based bait is irresponsive to the species, possibly due to the lack of palatability and biological variation. Recently, Neoh *et al.* (2011) has successfully eliminated *G. sulphureus* colonies using termite baiting. However, the authors claims that the baiting duration is remarkably longer (appro. 3 mo.) than those for managing lower termites. It is believed that the balls of vegetation that served as food store may delay the delivery of toxicant among colony members. Thus, this study was conducted to measure, analyze and compare the nutrient and organic matter of the nesting material of *G. sulphureus* (inner and outer mound part) and the adjacent soil around the mound as a comparison to observe how the termites modify the soil properties. The outcome of this study reveals some light into the chemical contents of *G. sulphureus* nest carton.

3.2. Methodology

3.2.1. Sampling of mound cartons

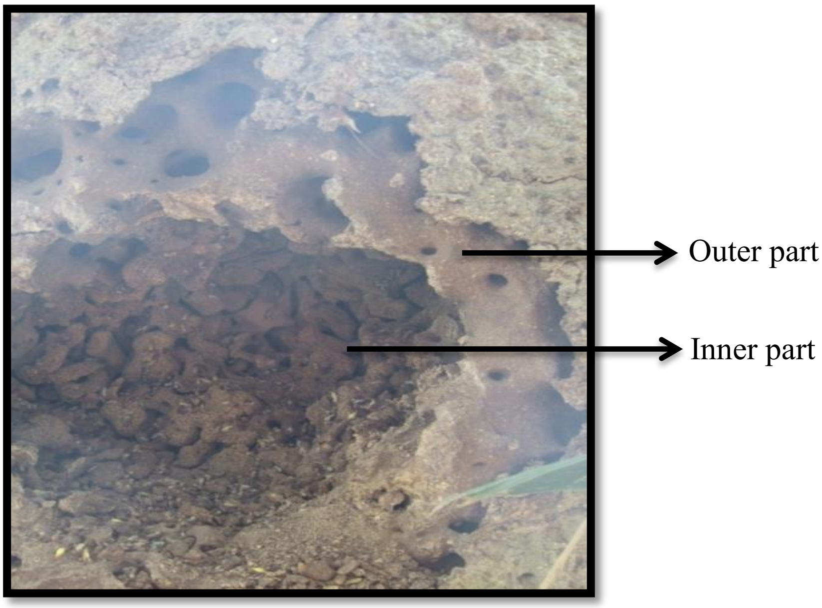
Sampling of *G. sulphureus'* nest materials was carried out at two locations in Penang, in the grassy land and the city. A total of 10 mounds were selected from each location (3 mounds from USM main campus; 5° 21.200' N, 100° 17.773' E, 2 mounds from Teluk Bahang; 5° 27.617' N, 100° 12.311 E, 2 mounds from Bayan Lepas; 5° 18.702 N, 100° 16.410 E and 3 mounds from Batu Uban; 5° 21.204 N, 100° 18.669 E). The height and bottom ciscumstance (width) of the mounds were measured and recorded. The dimensions of the mounds are presented in Table 3.1.

Table 3.1. Dimensions of the *G. sulphureus* mounda at various sites

No	Site		Height (cm)	Width (cm)
1	City (n=5)	1st mound	40	38
		2nd mound	55	51
		3rd mound	64	54
		4th mound	41	43
		5th mound	49	58
3	Forest (n=2)	1st mound	43	37
		2nd mound	68	42
4	Grass (n=3)	1st mound	54	46
		2nd mound	51	48
		3rd mound	57	52

Globitermes sulphureus mound was dug around the perimeter until the mound can be removed or tilted and the inner part of the mound were exposed. Samples were taken at two sections of the mound; inner and outer parts of the mound (Plate. 3.1). The inner materials were taken from the second layer of the nest, 25-30 cm from the external wall, in which it was dark brown in coloured, and located near to the royal chamber. The outer materials were taken from the fourth layer of the nest (internal wall). It was 5-7 cm from the external wall and characterized by its dark colour and high level of hardness. The termite cartons were taken from two layer of the mound section due to limited number of mound were found. The adjacent soils were taken at 10-20 cm away from each mound. Three sections of circumference of the mound were taken from as depth of 5-15 cm.

A



B

Plate 3.1 *G. sulphureus'* mound sampling section. (A) Mound of *G. sulphureus* (B) Sampling section and Inner structure of *G. sulphureus* mound